connectivity range predicted by a number of proxies in other parts of the world, such as current flows, spread of invasive species and patterns of genetic similarity [16]. Such data underpin the now widely-held rule of thumb that marine reserves placed a few tens of kilometres apart will exchange larvae of a wide range of species [17].

That there are no surprises in these findings should not for a moment detract from the importance of this study [1]. What it shows, put simply, is that the theoretical underpinnings of the use of marine reserves in both fisheries management and conservation are correct. They provide firm support for the efforts of thousands of people around the world who are creating protected areas to safeguard biodiversity and sustain the livelihoods of those dependent on fishing. They also hold a lesson: benefits from reserves are proportional to the build-up in the populations they support, which is dependent on the level of protection. It is salutary that in the new study, just four weeks of fishing by researchers and volunteers was enough to catch a quarter of all coral trout in reserves and a third of stripey snapper; reserves benefit top predators only at the highest levels of protection and populations take years to build. These benefits can be dissipated quickly by targeted fishing. Therefore, high levels of protection and resolute enforcement will produce the greatest benefits.

If Herubel's work had been heeded at the time, the world's oceans would be in a better state today as there would be many more marine protected areas. But as he lamented at the time, "…the exigencies of theory often accord ill with corporate interests, and the multiplication of coastal reserves would quickly arouse the anger of fishers" [1]. Those words remain true today as the fishing industry often vigorously opposes marine reserves. But Harrison *et al*. show that such opposition is misplaced: the industry has much to gain from protected areas.

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# Tubulogenesis: Src42A Goes to Great Lengths in Tube Elongation

**New work shows the instructive role of Src42A kinase in tube size regulation. By inducing polarized cell-shape changes, Src42A promotes tube elongation in the *Drosophila* tracheal system.**

**Amanda Ochoa-Espinosa, Magdalena M. Baer, and Markus Affolter***

The function of many essential organs, such as the lung, the kidney and the vascular system, depends on the correct size and shape of epithelial or endothelial tubes. Thus, it does not come as a surprise that several human pathologies are associated with tube-size defects. For example, polycystic kidney disease results in cystic overgrowth of the proximal and distal tubules and collecting ducts of the kidney [1], and stenotic tubes disturb the function of blood vessels [2]. However, it is still not well understood how tube growth and size dynamics are regulated during normal development and disease, and how individual cells within a given tube participate in these processes.

A well-established model to study the mechanisms controlling growth of biological tubes is the *Drosophila* respiratory system, the tracheae [3]. Several recent findings have helped us begin to understand how tube diameter and length are controlled (Figure 1). For instance, the COPI/COPII secretion apparatus is needed for initial lumen inflation, and further diametric

expansion and the deposition of a chitin cable allows for uniform growth in diameter. Furthermore, several factors have been identified that restrict tube elongation: septate junctions limit apical domain growth, probably by organizing polarity proteins, and the putative chitin-modifier proteins Serpentine and Vermiform structure the chitin cable to prevent tube over-elongation [4,5]. Given the relatively broad knowledge of tube length restriction and diameter expansion, it is surprising how little is known about how tracheal tubes elongate in the first place during embryonic development. It has been shown that the finer tracheal branches — for example, the dorsal branches — elongate due to stalk-cell intercalation and elongation driven by pulling forces generated by migrating tip cells [6]. However, our knowledge about the elongation of the main tracheal tube, the dorsal trunk, which traverses the embryo from anterior to posterior, is very poor. This situation has been changed by two recent papers in *Nature Cell Biology* from Förster and Luschnig [7] and Nelson *et al.* [8] identifying the Src42A protein (one of two fly Src homologs) as a key player promoting tube elongation.

Vertebrate Src non-receptor tyrosine kinases are expressed in a wide variety of cells and have been shown to play crucial roles in several cell biological processes, such as cell migration, cell-shape changes, and cell–substratum and cell–cell interactions. Src proteins are known to phosphorylate several proteins associated with the cytoskeleton and adherens junctions [9]. *Drosophila* has two genes encoding Src homologues (*Src64* and *Src42A* [10]) and the new reports characterize the tracheal defects in embryos carrying a defective *Src42A* gene. The phenotype observed concerns the main branch of the tracheal system, the dorsal trunk. *Src42A* mutants have a significantly shorter dorsal trunk with a wider lumen in a normally shaped and proportioned embryo (Figure 1A). Up to date, this phenotype is unique since previously identified mutations in different pathways affecting dorsal trunk length have only been reported to give rise to longer tubes.

To better understand how Src42A regulates tracheal development, it was essential to first characterize the normal course of tube elongation. To this end Förster and Luschnig [7] took a live-imaging approach and followed dorsal trunk cells during the elongation phase. The expression of an α-Catenin–GFP fusion protein in the tracheae allowed them to outline the apical surfaces of individual cells. By quantifying the apical area of cells, they observed that, in the wild-type dorsal trunk, cells rearrange and expand both circumferentially and axially, which ultimately leads to an increase in tube length and diameter.

These elegant live-imaging analyses of dorsal trunk development provided an excellent context for the analysis of the *Src42A* phenotype and both groups, through different approaches, find that the apical surface of mutant cells is significantly reduced and that the longest surface axial extension of these cells is biased towards the circumferential axis, in contrast to wild-type embryos, in which surface expansion is more broadly distributed (Figure 1A). Strikingly, the live-imaging approach allowed Förster and Luschnig [7] to observe that *Src42A* mutant apical cell profiles expanded more slowly in all directions; however, only the circumferential junctions extend completely, leaving zigzag-like axially oriented adherens junctions. These observations led the two groups to conclude that Src42A-dependent anisotropic expansion along the longitudinal axis is a main driving force for elongation, and that Src42A is also required for overall apical expansion.

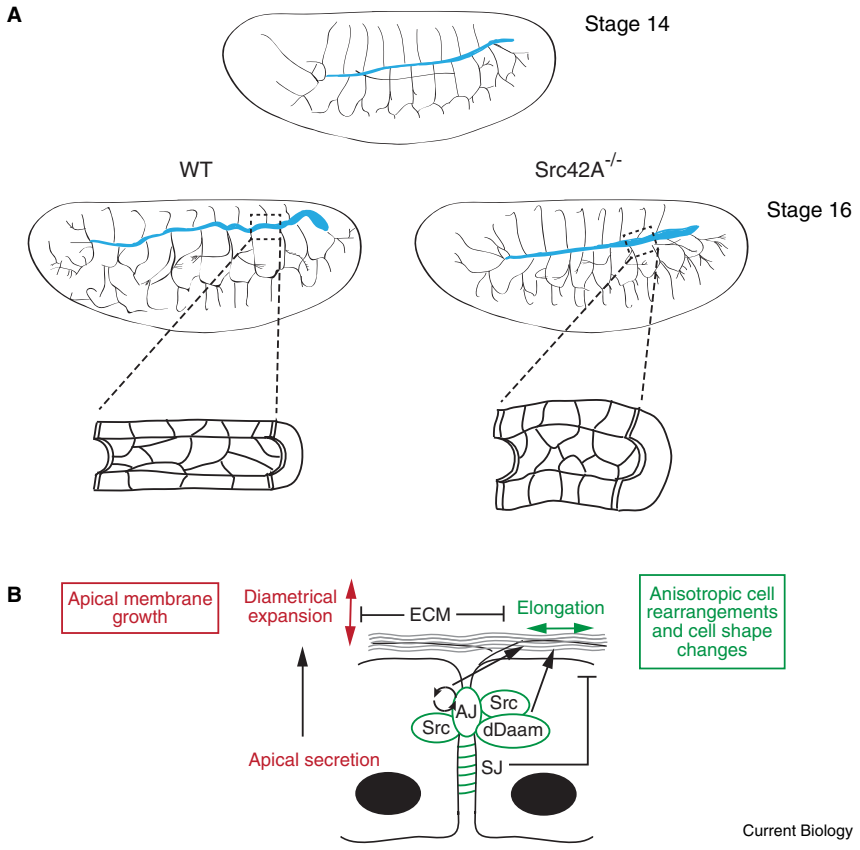


Figure 1. A model for tracheal tube expansion control in *Drosophila*.

(A) The *Drosophila* dorsal trunk (blue) starts to elongate at mid-embryogenesis. In the absence of Src42A this process is disrupted, leading to a short dorsal trunk phenotype. (B) Growth of tracheal tubes during embryogenesis involves expansion both in length and in diameter. Elongation of the tubes is initiated by Src42A-dependent cell rearrangements and anisotropic growth of the apical surface, whereas apical extracellular matrix (ECM) and septate junction (SJ) proteins are required to prevent tube over-elongation. Src42A mediates cell shape changes by inducing adherens junction (AJ) remodeling; at the same time, Src42A promotes axially oriented expansion of apical surfaces together with dDaam/formin. These events lead to a preferential increase of tube length at the expense of tube diameter; circumferential expansion is achieved via secretion-dependent membrane growth in a Src42A-independent manner.

Src42A is necessary and sufficient for the elongation of tracheal cells along the longitudinal axis and its kinase activity is required for this role. The function of Src42A in the trachea is cell autonomous, as elegantly demonstrated by Förster and Luschnig [7], who showed that expressing *Src42A* in just three body segments rescued the *Src42A* phenotype only in the corresponding tracheal metamers — the repetitive subunits that compose the tracheal system at each embryonic body segment. Importantly, Nelson *et al.* [8] find that overall phosphotyrosine levels are severely reduced in *Src42A* mutants, suggesting that phosphotyrosine signaling in the tracheae is, to a large extent, under the control of Src42A. Furthermore, both groups confirm that, while endogenous Src42A localizes throughout the plasma membrane, activated Src42A colocalizes with E-cadherin at adherens junctions [11].

But at what level does Src42A act to control tube size? Several genes are known to affect the dimensions of tracheal tubes. They can be generally grouped into three functional classes: proteins involved in apical–basal polarity, proteins involved in septate-junction assembly [12], and proteins involved in apical extracellular matrix deposition [5]. Proteins of these three classes function to restrict tube elongation and, when absent, give rise to an over-elongation phenotype. The cellular localization of these components remained undisturbed in *Src42A* mutants. The short dorsal trunk phenotype of *Src42A* mutants was epistatic to several genes encoding components of each of these categories, and the over-elongation phenotype observed in these mutants was not due to increased expression or activation of Src42A. All of these findings suggest that Src42A acts in parallel to, or downstream of, these pathways to control tube elongation.

Although the dorsal trunk epithelium shows a low level of planar polarization along the anterior-posterior axis, there is no evidence that the planar cell polarity (PCP) pathway functions in its canonical way in the tracheal system (i.e. its components appear to be uniformly localized along junctional domains and mutations in PCP components produce only mild over-elongation phenotypes [13]). Consistent with these observations, no connection to the PCP pathway was observed in the two studies, and the short dorsal trunk phenotype of *Src42A* was epistatic to the over-elongation phenotype of PCP genes, while the over-elongation in PCP mutants was not accompanied by increased Src42A levels or activation. So Src42A appears to work independently of PCP to regulate tube elongation.

Lastly, Förster and Luschnig [7] tested the potential interactions of *Src42A* and the known components of the secretion apparatus, which have recently been shown to control tube-diameter expansion. Double-mutant embryos lacking *Src42A* and *sten* (*Sec24*), which affects luminal secretion, had small apical domains and short dorsal trunks. Interestingly, constitutive activation of Src42A in *sten* mutants caused an over-elongation phenotype, arguing that the ability of Src42A to induce tube elongation does not depend on the secretory apparatus and that circumferential and axial expansion are regulated by distinct cellular processes. These results are consistent with the distinct kinetics of circumferential and axial expansion of the tracheae [14].

In order to obtain more insight into the possible molecular mechanism by which Src42A regulates tube elongation, both groups decided to analyze the relationship of Src and some of its interactors identified in other studies. Src42A is known to genetically and physically interact with adherens-junction components in *Drosophila* [11,15] and Förster and Luschnig [7] characterized the dynamics of endogenous E-cadherin in the embryonic epidermis of *Src42A* mutants. They found that, in the absence of Src42A, E-cadherin recycling at adherens junctions is affected, suggesting that defects in cell-shape changes in the *Src42A* mutants could in part be due to defective junction remodeling. The authors thus propose that dorsal trunk elongation is mediated by Src42A-dependent recycling of junction material followed by anisotropic cell-shape remodeling (Figure 1B).

Nelson *et al.* [8] followed the lead of the known interaction of vertebrate Src and Daam, a Diaphanous-related formin [16], and obtained evidence that these two proteins act together in controlling directional apical growth during tube elongation. They showed that zygotic *dDaam* mutants have a short but thick dorsal trunk phenotype and, similarly to *Src42A* mutation, that the individual apical surfaces in this mutant are elongated preferentially along the circumferential axis, while the overall apical area remained unchanged. Additionally, dDaam colocalizes and physically interacts with active Src42A and seems to act upstream of Src42A. Based on their observations Nelson *et al.* [8] propose that directional (axial) apical surface growth is controlled jointly by dDaam and Src42A (Figure 1B). It would be interesting to find out whether Src42A-dependent E-cadherin recycling is also regulated by interactions with dDaam.

Many questions remain regarding the role of Src42A in tracheal tube elongation. Given that Src42A is responsible for most of the tyrosine phosphorylation at adherens junctions and apical membranes in tracheal cells, what are its specific targets in the process of elongation? Also, what provides the directional cues along the tube axis? Förster and Luschnig [7] propose that the physical constraints of cylindrical geometry could be sensed by Src42A, which then translates them into polarized cell behavior [17,18].

The tracheal system serves as a model for many aspects of branching morphogenesis, and the novel role of Src42A in promoting anisotropic apical surface expansion resulting in organ elongation provides a starting point to understand how individual cell behaviors influence the development of entire organs. Furthermore, these studies may point to possible scenarios through which Src kinases regulate the development of other tubular organs in which they have been implicated, such as the vasculature [19] and the kidney [20].

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# Tumour Invasion: A New Twist on Rac-Driven Mesenchymal Migration

**Elongated mesenchymal migration of cancer cells is driven by Rac1 activation mediated by the adaptor NEDD9 and the exchange factor DOCK3. A new study reports a role for the transcription factor Twist1 in inducing mesenchymal migration by relieving the suppression of NEDD9 and DOCK3 by the microRNA let-7i.**

**Victoria Sanz-Moreno**

The epithelial–mesenchymal transition (EMT) is a highly conserved and fundamental process that governs morphogenesis in multicellular organisms and is thought to promote metastatic progression of carcinomas [1]. Some key events typical of EMT are loss of the cell–cell adhesion molecule E-cadherin, gain of N-cadherin and augmented expression of certain transcription factors. EMT leads to loss of cell–cell adhesion and increased cell migration and invasion [1]. Twist1, a transcriptional regulator, induces EMT by suppression of E-cadherin [2]. A recent paper by Yang *et al.* [3] now reveals that Twist1 is a key regulator of elongated mesenchymal migration of cancer cells through regulation of a microRNA (miRNA) that controls signalling by the Rho-family GTPase Rac.

Rho-family GTPases are key regulators of cell migration through their actions on actin assembly and actomyosin contractility. Cells can migrate as collective groups or as individual cells. Two modes of individual cell migration have been characterized in a number of systems. An elongated ('mesenchymal-like') mode is characterized by cell polarization, a requirement for extracellular proteolysis [4], and low actomyosin contractility [5], and is driven by the formation of membrane protrusions that result from a localized activation of the Rac GTPase [6]. Rounded 'amoeboid' modes of cell migration are driven by high levels of actomyosin contractility regulated by Rho–ROCK signalling [5,7] or the Cdc42 GTPase [8]. In rounded moving cells, high hydrostatic forces drive cell movement resulting in membrane blebbing [9]. Interestingly, Cdc42 is capable of regulating elongated mesenchymal or rounded contractile movement via usage of different guanine nucleotide exchange factors (GEFs) [8].

Elongated mesenchymal movement can be driven by activation of Rac1 through the GEF DOCK3 complexed with the adaptor protein NEDD9 [5]. Rac1 promotes elongation through WAVE2-mediated actin polymerization [5,10,11]. Furthermore, Rac1 signalling suppresses rounded movement [5,11,12] through decreasing actomyosin contractility [5,12]. In an effort to identify new functions of Twist1, Yang *et al.* [3] screened for possible new targets with a particular focus on miRNAs. They reasoned that Twist1 cooperates with the Polycomb group protein BMI1 [2] and they selected head and neck squamous cell carcinoma (HNSCC) cell lines that would have different expression levels of the Twist1–BMI1 pairing to compare their migratory behaviour. The authors performed microarray analysis to identify which miRNAs were co-regulated by both Twist1 and BMI1 and found that these proteins co-repressed let-7i miRNA [3]. The authors confirmed that loss of let-7i induced a morphological switch into a mesenchymal program of invasion [3], with long-lived protrusions that are typical of a Rac-driven phenotype. They went on to find that let-7i downregulated NEDD9 and DOCK3 [3], both of which are activators of Rac and drive mesenchymal movement in several systems [3,5,10,12]. Furthermore, Twist1 overexpression induced Rac1 activation in HNSCCs, as a result of increased expression of both NEDD9 and DOCK3 [3] (Figure 1). Interestingly, other miRNAs have been shown to regulate mesenchymal migration: miR-200 family members can regulate the plasticity of tumour cell movement [13] and the miR-200c target MARCKS is capable of regulating mesenchymal invasion by driving cell protrusions [13].